Metabolomic Analyses in Active Duty Military Participants

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Abstract

Active duty military personnel exposed to traumatic warzone events are at an increased risk for developing post-traumatic stress disorder (PTSD). Pre-deployment identification of risk/ resilience factors is crucial in developing strategies to reduce or prevent PTSD symptoms. Comorbidities of combat PTSD with cardiovascular disease, metabolic syndrome, and other diseases suggest that there are significant metabolic differences between subjects with combat-related PTSD and controls. The Fort Campbell Cohort Study is a prospective longitudinal cohort study designed to accelerate the development of sensitive and specific pre-deployment biological markers[1] to aid in the prediction and diagnosis of PTSD. In this study, we investigated the biological consequences of combat elicited PTSD in a longitudinal manner based on blood high-throughput metabolomics. Data of active duty Army personnel was collected prior to deployment (N=1,029), three days after a 10-month deployment (N=760) and 90-180 days post-deployment (N=1,166). Plasma metabolomic analysis (N = 1400) was carried out by Metabolon. The Active Duty was further divided into four categories (PTSD (N=146)), PTSD subthreshold (Sub, N =171), High resilience group, (HRG, N=502) and Low resilience group (LRG, N= 505)) based on their psychological scores. Both PTSD and subthreshold group have significantly higher Glycolytic ratio than HRG (pv = 0.0004 and 0.0013), while both groups have a significantly lower global arginine bioavailability ratio (GABR) (pv =0.0019 and 0.0217). The PTSD group also has lower serotonin (pv = 0.0083) and higher glutamate (pv=0.0345) than high resilience group (HRG), which is clinically correlated to depressive and anxiety symptoms in the PTSD group. These metabolic differences were not explained by gender, age, body mass index, smoking, or uptake of energy drink/coffee. The findings have clinical implications for assessing pre-deployment factors predictive of PTSD risk and understanding the evolution of PTSD to inform pre-deployment resilience training.

Background

Combat deployment can involve drastic changes in activity, diet, and stress with strong metabolic consequences. Characterizing the metabolic dysregulation accompanying Post-Traumatic Stress Disorder (PTSD), could enhance our understanding, diagnosis, and treatment of PTSD. We collected a battery of clinical and psychological data as well as whole blood draws at pre-deployment, post-deployment, and at a three month follow-up from active duty soldiers.

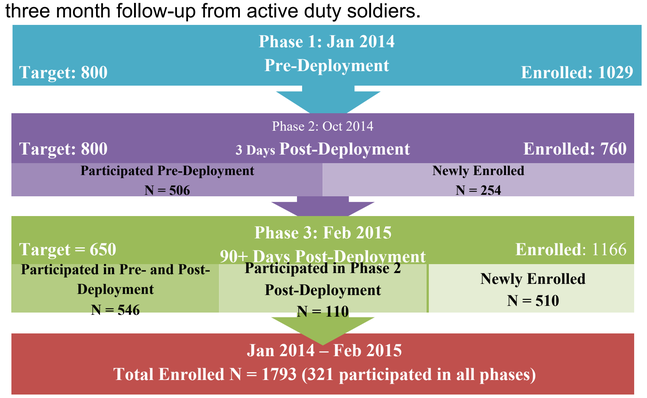

Figure 1. Sample collection for the Cohort

Methods

Blood from Active duty Army personnel was collected in EDTA tubes followed by plasma separation using manufacturer guidelines. The samples were collected once at pre-deployment and at multiple time-points following post-deployment. Samples were stored at -80˚C for global metabolomics assays.The samples were shipped to the Metabolon facility for analysis. For data analysis ,LC/MS, GC-FID raw peak values are scaled (median set to 1) and missing value imputed with the minimum. For each comparison, ratios given and appropriate statistical test done for each metabolite. Interpretation focused on pathway trends, not individual metabolites.

PTSD Checklist for DSM-5 (**PCL-5**; Weathers et al., 2013) Participants completed PCL-5 in response to an event they deemed to be the most stressful and life-threatening.. ***LRG -Low resilience group**- current PCL<=15, but the individual was diagnosed as PTSD or Subthreshold at any time point, or PSQI>=8, or PHQ>=10, or GAD>=10 at any time point.
***HRG-High resilience group** current PCL<=15, and not satisfied the criteria of LRG..
Subthreshold: 30>PCL>15 **PTSD:** PCL >=30

Results

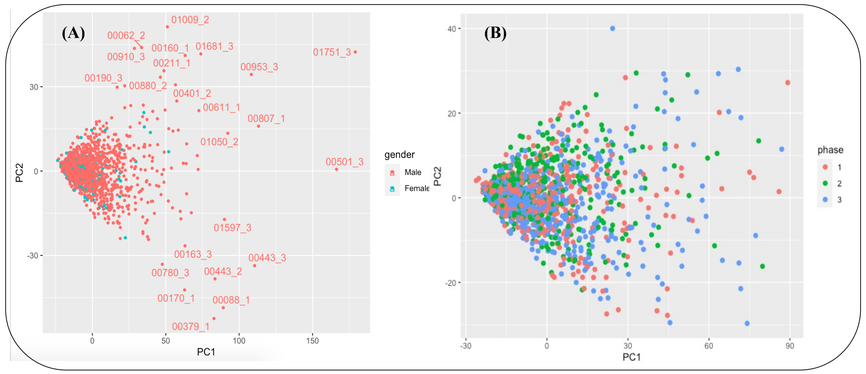

Figure 2. Data quality check A) outliers in the data B) Batch effects in the phases

- There were a total of 1582 metabolites with 985 lipid panel.
- 1400 samples had 450, 418 and 532 at phase 1, 2 and 3 respectively.
- There were 1235 male subjects and 165 female subjects
- After quality check, 1375 subjects with 1516 metabolites were proceeded for analysis
- There is no batch effects for the phase 1, 2 and 3

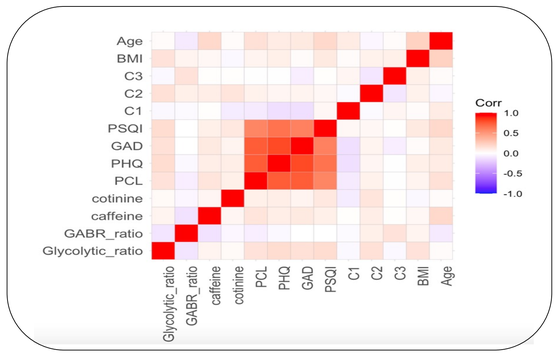

Figure 3. Correlation of Demographic factors and clinical Scores

Table 1. PTSD characteristics for the cohort using PCL score

Column1	Samples	Age	BMI	PCL >=30	30>PCL >15	PCL<=15	HRG	LRG	Sub	PTSD	Unknown
Phase 1	440	25.7 (5.5)	26.9 (3.8)	16	36	371	185	186	36	16	17
Phase 2	412	26.8 (5.9)	27.3 (3.2)	29	45	327	156	171	45	29	11
Phase 3	523	27.6 (6.2)	27.5 (3.8)	103	92	324	161	163	92	103	4

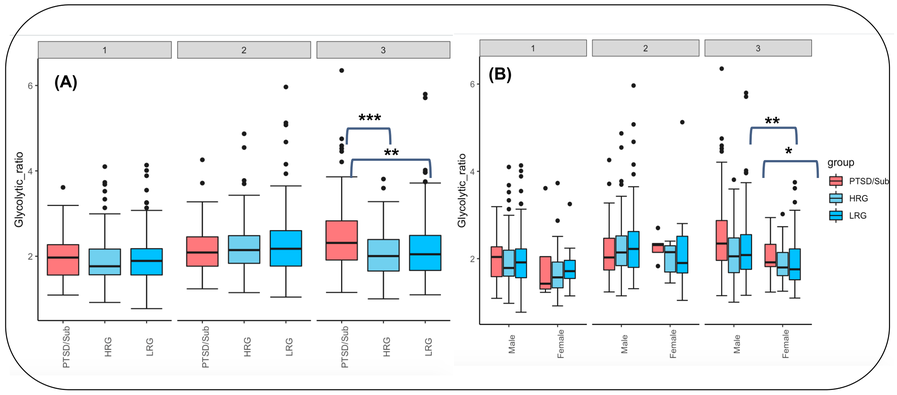

Figure 4. Glycolytic ratio in 1) response to PCL score groups 2) Gender related across phase 1, 2 and 3. p value=*<0.001 ** <0.01 * <0.05**

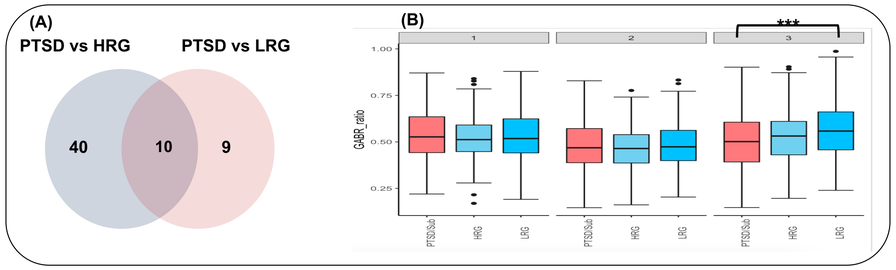

Figure 5. Differentially expressed metabolites when compared PTSD group is compared with HRG and LRG group. B) GABR (global arginine bioavailability ratio) in response to PCL score groups p value=*<0.001 ** <0.01 * <0.05**

Conclusion

- In previous studies using veterans cohort, pyruvate and lactate, two metabolites that are end-products of anaerobic respiration in glycolysis, were significantly elevated in veterans with PTSD and Citrate, a TCA cycle intermediate, was decreased in PTSD.
- In this study, Glutamate, lactate, and pyruvate, are increased in high PCL-5 vs lower PCL score when compared across phases and citrate is decreased in High PCL-5 vs Low PCL-5 at Phase 3.
- PTSD subgroup have higher Glycolytic ratio, DHEA-S, Glutamate, cotinine, Pyroglutamic acid , N-acetyl-L-tyrosine and lower serotonin than HRG subgroup. PTSD subgroup has lower GABR ratio than LRG subgroup
- PTSD has overexpressed Caffeine pathway than controls
- Subthreshold subgroup has higher Glycolytic ratio, DHEA-S, cotinine, Pyroglutamic acid and lower Oxalate than HRG subgroup. Subthreshold has lower GABR ratio than LRG subgroup.